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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/00 — A2

(11) International Publication Number: **WO 99/09151**

(43) International Publication Date: 25 February 1999 (25.02.99)

(21) International Application Number: PCT/US98/14841

(22) International Filing Date: 17 July 1998 (17.07.98)

(30) Priority Data:
08/910,386 13 August 1997 (13.08.97) US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: PROCEDURES AND MATERIALS FOR CONFERRING DISEASE RESISTANCE IN PLANTS

(57) Abstract

The present invention provides nucleic acids encoding polypeptides which confer resistance to *Xanthomonas* spp and other pathogens. The nucleic acids can be used to produce transgenic plants resistant to the pathogen.

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PROCEDURES AND MATERIALS FOR CONFERRING DISEASE RESISTANCE IN PLANTS

This application is related to U.S. Patent Application No. 08/587,680, filed January 17, 1996, which is a continuation in part of copending U.S. patent application No. 08/567,375, filed December 4, 1995, which is a continuation in part of U.S. provisional patent application No., 60/004,645. The '680 application is also a continuation in part of copending U.S. patent application No. 08/475,891, filed June 7, 1995, which is a continuation in part of copending U.S. patent application No. 08/373,374, filed January 17, 1995. These applications are incorporated herein by reference.

Field Of The Invention

The present invention relates generally to plant molecular biology. In particular, it relates to nucleic acids and methods for conferring disease resistance in plants.

Statement as to Rights to Inventions Made Under Federally Sponsored Research and Development

This invention was made with Government support under Grant No. GM47907, awarded by the National Institutes of Health and Grant No. 9300834, awarded by the United States Department of Agriculture. The Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Loci conferring disease resistance have been identified in many plant species. Genetic analysis of many plant-pathogen interactions has demonstrated that plants contain loci that confer resistance against specific races of a pathogen containing a complementary avirulence gene. Molecular characterization of these genes should provide means for conferring disease resistance to a wide variety of crop plants.

Those plant resistance genes that have been characterized at the molecular level fall into four classes. One gene, *Hm1* in corn, encodes a reductase and is effective against the fungal pathogen *Cochliobolus carbonum* (Johal *et al. Science* 258:985-987 (1992)). In tomato, the *Pto* gene confers resistance against *Pseudomonas syringae* that express the *avrPto* avirulence gene (Martin *et al. Science* 262:1432 (1993)). The predicted *Pto* gene encodes a serine threonine protein kinase. The tomato *Cf-9* gene confers resistance to races of the fungus *Cladosporium fulvum* that carry the avirulence gene *Avr9* (Jones *et al. Science* 266:789- 793 (1994). The tomato *Cf-9* gene encodes a putatitive extracellular LRR protein. Finally, the *RPS2* gene of *Arabidopsis thaliana* confers resistance to *P. syringae* that express the *avrRpt2* avirulence gene (Bent *et al. Science* 265:1856-1860 (1994)). *RPs2* encodes a protein with an LRR motif and a P-loop motif.

Bacterial blight disease caused by *Xanthomonas* spp. infects virtually all crop plants and leads to extensive crop losses worldwide. Bacterial blight disease of rice (*Oryza sativa*), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is an important disease of this crop. Races of *Xoo* that induce resistant or susceptible reactions on rice cultivars with distinct resistance (*Xa*) genes have been identified. One source of resistance (*Xa21*) had been identified in the wild species *Oryza longistaminata* (Khush *et al. in Proceedings of the International Workshop on Bacterial Blight of Rice.* (International Rice Research Institute, 1989) and Ikeda *et al. Jpn J. Breed* 40 (Suppl.1):280-281 (1990)). *Xa21* is a dominant resistance locus that confers resistance to all known isolates of *Xoo* and is the only characterized *Xa* gene that carries resistance to *Xoo* race 6. Genetic and physical analysis of the *Xa21* locus has identified a number of tightly linked markers on chromosome 11 (Ronald *et al. Mol. Gen. Genet.* 236:113-120 (1992)). The molecular mechanisms by which the *Xa21* locus confers resistance to this pathogen were not identified, however.

Considerable effort has been directed toward cloning plant genes conferring resistance to a variety of bacterial, fungal and viral diseases. Only one pest resistance gene has been cloned in monocots. Since monocot crops feed most humans and animals in the world, the identification of disease resistance genes in these plants is particularly important. The present invention addresses these and other needs.

SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acid constructs comprising an *RRK* polynucleotide sequence. The sequences can be rice sequences which hybridize to SEQ ID NOs: 1, 4, 6, 8, 10, or 11 under stringent conditions. Also claimed are sequences from cassava which hybdridize to SEQ ID NO: 13), maize sequences which hybridize to SEQ ID NOs: 15, 16), and tomato (*e.g.*, SEQ ID NOs:17, 19, or 21). Exemplary *RRK* polynucleotide sequences are *Xa21* sequences which encode an *Xa21* polypeptide as shown below. The RRK polynucleotides encode a protein having a leucine rich repeat motif and/or a cytoplasmic protein kinase domain. The nucleic acid constructs of the invention may further comprise a promoter operably linked to the *RRK* polynucleotide sequence. The promoter may be a tissue-specific promoter or a constitutive promoter.

The invention also provides nucleic acid constructs comprising a promoter sequence from an *RRK* gene linked to a heterologous polynucleotide sequence. Exemplary heterologous polynucleotide sequences include structural genes which confer pathogen resistance on plants.

The invention further provides transgenic plants comprising a recombinant expression cassette comprising a promoter from an *RRK* gene operably linked to a polynucleotide sequence as well as transgenic plants comprising a recombinant expression cassette comprising a plant promoter operably linked to an *RRK* polynucleotide sequence. Although any plant can be used in the invention, rice and tomato plants may be conveniently used.

The invention further provides methods of enhancing resistance to *Xanthomonas* and other pathogens in a plant. The methods comprise introducing into the plant a recombinant expression cassette comprising a plant promoter operably linked to an RRK polynucleotide sequence. The methods may be conveniently carried out with rice or tomato plants.

Definitions

The term "plant" includes whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds and plant cells and progeny of same. The class of plants which can be used in the methods of the invention is generally as broad as the class of higher plants

amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants.

A "heterologous sequence" is one that originates from a foreign species, or, if from the same species, is substantially modified from its original form. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived, or, if from the same species, one or both are substantially modified from their original form.

An "*RRK* gene" is member of a new class of disease resistance genes which encode RRK polypeptides which typically comprise an extracellular LRR domain, a transmembrane domain, and a cytoplasmic protein kinase domain (as shown in *e.g.*, *Pto* and *Fen* (Martin *et al. Plant Cell* 6:1543-1552 (1994)). As used herein, an LRR domain is a region of a repeated unit of about 24 residues as described in USSN 08/587,680, and found in *Cf-9*). Using the sequences disclosed here and standard nucleic acid hybridization and/or amplification techniques, one of skill can identify members of this class of genes. For instance, a nucleic acid probe from an *Xa21* gene detected polymorphisms that segregated with the blast (*Pyricularia oryzae*) resistance gene (Pi7) in 58 recombinant inbred lines of rice. The same probe also detected polymorphism in nearly isogenic lines carrying *xa5* and *Xa10* resistance genes.

In some preferred embodiments, members of this class of disease resistance genes can be identified by their ability to be amplified by degenerate PCR primers which correspond to the LRR and kinase domains. For instance, primers have been used to isolate homologous genes in tomato, maize and cassava. The maize gene disclosed here has been genetically mapped to a region associated with resistance to *Helminthosporium turcicum*. Exemplary primers for this purpose are tcaagcaacaatttgtcaggnca (a/g) at (a/c/t) cc (for the LRR domain sequence GQIP) and taacagcacattgcttgatttnan (g/a) tcncg (g/a) tg (the kinase domain sequence HCDIK). These or equivalent primers are then used to amplify the appropriate nucleic acid using the PCR conditions described below.

An "*Xa21* polynucleotide sequence" is a subsequence or full length polynucleotide sequence of an *Xa21* gene, such as the rice *Xa21* gene, which, when present in a transgenic plant confers resistance to *Xanthomonas* spp. (*e.g.*, *X. oryzae*) on the plant. Exemplary polynucleotides of the invention include the coding region of the sequences provided below. An *Xa21* polynucleotide is typically at least about 3100

nucleotides to about 6500 nucleotides in length, usually from about 4000 to about 4500 nucleotides.

An "*Xa21* polypeptide" is a gene product of an *Xa21* polynucleotide sequence, which has the activity of *Xa21*, *i.e.*, the ability to confer resistance to *Xanthomonas spp*. *Xa21* polypeptides, like other *RRK* polypeptides, are characterized by the presence of an extracellular domain comprising a region of leucine rich repeats (LRR) and/or a cytoplasmic protein kinase domain. Exemplary *Xa21* polypeptides of the invention include those described below.

In the expression of transgenes one of skill will recognize that the inserted polynucleotide sequence need not be identical and may be "substantially identical" to a sequence of the gene from which it was derived. As explained below, these variants are specifically covered by this term.

In the case where the inserted polynucleotide sequence is transcribed and translated to produce a functional *RRK* polypeptide, one of skill will recognize that because of codon degeneracy, a number of polynucleotide sequences will encode the same polypeptide. These variants are specifically covered by the term "*RRK* polynucleotide sequence". In addition, the term specifically includes those full length sequences substantially identical (determined as described below) with an *RRK* gene sequence and that encode proteins that retain the function of the *RRK* protein. Thus, in the case of rice *RRK* genes disclosed here, the above term includes variant polynucleotide sequences which have substantial identity with the sequences disclosed here and which encode proteins capable of conferring resistance to *Xanthomonas* or other plant diseases and pests on a transgenic plant comprising the sequence.

Two polynucleotides or polypeptides are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below. The term "complementary to" is used herein to mean that the complementary sequence is identical to all or a portion of a reference polynucleotide sequence.

Sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a segment or "comparison window" to identify and compare local regions of sequence similarity. The segment used for purposes of comparison may be at least about 20

contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

"Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 60% sequence identity, preferably at least 80%, more preferably at least 90% and most preferably at least 95%, compared to a reference sequence using the programs described above (preferably BESTFIT) using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 40%, preferably at least 60%, more preferably at least 90%, and most preferably at least 95%. Polypeptides which are "substantially similar" share sequences as noted above except that residue positions which are not identical may differ by conservative amino acid changes. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For

example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under appropriate conditions. Appropriate conditions can be high or low stringency and will be different in different circumstances. Generally, stringent conditions are selected to be about 5°C to about 20°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent wash conditions are those in which the salt concentration is about 0.02 molar at pH 7 and the temperature is at least about 60°C. However, nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This may occur, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. For Southern hybridizations, high stringency wash conditions will include at least one wash in 0.1X SSC at 65°C.

Nucleic acids of the invention can be identified from a cDNA or genomic library prepared according to standard procedures and the nucleic acids disclosed here (typically at least 100 nucleotides to about full length) used as a probe. Low stringency hybridization conditions will typically include at least one wash using 2X SSC at 65°C. The washes are preferrably followed by a subsequent wash using 1X SSC at 65°C.

As used herein, a homolog of a particular *RRK* gene (*e.g.*, the rice *Xa21* genes disclosed here) is a second gene (either in the same species or in a different species) which encodes a protein having an amino acid sequence having at least 25% identity or 45% similiarity to (determined as described above) to a polypeptide sequence in the first

gene product. It is believed that, in general, homologs share a common evolutionary past.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the genome organization of the seven *Xa21* family members and location of 14 transposon-like elements. Cosmid and BAC clones carrying the family members are designated. Wide bars represent predicted coding regions, fine bars represent noncoding regions, introns are indicated by angled lines, and the non-sequenced regions are shown by straight lines. A gap in the sequence of BAC9 is indicated by "//". Letters refer to names of *Xa21* gene family members and arrows indicate direction of ORFs. The 14 transposon-like elements are numbered and represented by closed triangles.

Figure 2A shows the HC region of the sequenced *Xa21* gene family members. Wide bars represent predicted coding regions, and fine bars represent non-coding regions. Start and stop codons are indicated. The 5' flanking regions and downstream regions are grouped into four and two groups, respectively, and are shown in different colors based on sequence identity. The percentage of DNA sequence identity between promoter regions and between classes is shown to the left and right, respectively. The HC region is indicated by a black bar.

Figure 2B is a schematic diagram showing a comparison of the predicted amino acid sequences of XA21 and A1. Domains are numbered as follows: I, Presumed signal peptide; II, presumed N terminus; III, LRR; VI, charged; V, presumed transmembrane; VI charged; VII juxtamembrane; VIII, serine/threonine kinase; IX, carboxy tail. The numbers below each domain indicate amino acid identity between XA21 and A1.

Figure 3A shows family member D and insertion position of *Retrofit*. *Retrofit* carries long terminal repeats (LTRs) (small arrows) and a single, large ORF, encoding a protein with the following domains: gag, protease (PR), integrase (IN), reverse transcriptase (RT), and RNase H (RH). The large arrow indicates direction of the ORF.

Figure 3B shows family member E and insertion position of *Truncator*. Arrows mark the orientation of the inverted repeats. The deduced amino acid sequences of the tomato resistance genes Cf9 and Pto are shown below. In both Figures 3A and 3B, the insertion elements are designated by a hatched bar. The presumed deduced amino acid sequences of members D and E are shown by shaded rectangles. Domains representations

are as described in the legend to Figure 2.

Figure 4 shows intergenic recombination break point in the *Xa21* family members. Boxes represent the ORFs of the designated family members, while narrow boxes represent flanking regions. Same colors indicate a high level of sequence homology. The nucleotides of the presumed recombination break points are indicated in large and bold type. Sequences surrounding the recombination break point are also shown.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

This invention relates to plant *RRK* genes, such as the *Xa21* genes of rice. Nucleic acid sequences from *RRK* genes, in particular *Xa21* genes, can be used to confer resistance to *Xanthomonas* and other pathogens in plants. The invention has use in conferring resistance in all higher plants susceptible to pathogen infection. The invention thus has use over a broad range of types of plants, including species from the genera *Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Ciahorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Zea, Avena, Hordeum, Secale, Triticum,* and, *Sorghum.*

The Example section below, which describes the isolation and characterization of *RRK* genes in rice, casava, maize and tomato. The methods used to isolate these genes are exemplary of a general approach for isolating *Xa21* genes and other *RRK* genes. The isolated genes can then be used to construct recombinant vectors for transferring *RRK* gene expression to transgenic plants.

Generally, the nomenclature and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art. Standard techniques are used for cloning, DNA and RNA isolation, amplification and purification. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications. These techniques and various other techniques are generally performed

according to Sambrook *et al.*, *Molecular Cloning - A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, (1989).

The isolation of *Xa21* and related *RRK* genes may be accomplished by a number of techniques. For instance, oligonucleotide probes based on the sequences disclosed here can be used to identify the desired gene in a cDNA or genomic DNA library. To construct genomic libraries, large segments of genomic DNA are generated by random fragmentation, e.g. using restriction endonucleases, and are ligated with vector DNA to form concatemers that can be packaged into the appropriate vector. To prepare a cDNA library, mRNA is isolated from the desired organ, such as leaf and a cDNA library which contains the *RRK* gene transcript is prepared from the mRNA. Alternatively, cDNA may be prepared from mRNA extracted from other tissues in which RRK genes or homologs are expressed.

The cDNA or genomic library can then be screened using a probe (typically a degenerate probe) based upon the sequence of a cloned *RRK* gene such as rice *Xa21* genes disclosed here. Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different plant species.

Alternatively, the nucleic acids of interest can be amplified from nucleic acid samples using amplification techniques. For instance, polymerase chain reaction (PCR) technology to amplify the sequences of the *RRK* and related genes directly from genomic DNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes.

Appropriate primers and probes for identifying *RRK* sequences from plant tissues are generated from comparisons of the sequences provided herein. For a general overview of PCR see *PCR Protocols: A Guide to Methods and Applications*. (Innis, M, Gelfand, D., Sninsky, J. and White, T., eds.), *Academic Press*, San Diego (1990), incorporated herein by reference.

Polynucleotides may also be synthesized by well-known techniques as described in the technical literature. See, e.g., Carruthers *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* 47:411-418 (1982), and Adams *et al.*, *J. Am. Chem. Soc.* 105:661

(1983). Double stranded DNA fragments may then be obtained either by synthesizing the complementary strand and annealing the strands together under appropriate conditions, or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Isolated sequences prepared as described herein can then be used to provide *RRK* gene expression and therefore *Xanthomonas* resistance in desired plants. One of skill will recognize that the nucleic acid encoding a functional *RRK* protein need not have a sequence identical to the exemplified gene disclosed here. In addition, the polypeptides encoded by the *RRK* genes, like other proteins, have different domains which perform different functions. Thus, the *RRK* gene sequences need not be full length, so long as the desired functional domain of the protein is expressed. As explained in detail below, the proteins of the invention comprise an extracellular leucine rich repeat domain, as well as an intracellular kinase domain. Modified protein chains can also be readily designed utilizing various recombinant DNA techniques well known to those skilled in the art. For example, the chains can vary from the naturally occurring sequence at the primary structure level by amino acid substitutions, additions, deletions, and the like. Modification can also include swapping domains from the proteins of the invention with related domains from other pest resistance genes. For example, the extra cellular domain (including the leucine rich repeat region) of the proteins of the invention can be replaced by that of the tomato *Cf-9* gene and thus provide resistance to fungal pathogens of rice. These modifications can be used in a number of combinations to produce the final modified protein chain.

To use isolated *RRK* sequences in the above techniques, recombinant DNA vectors suitable for transformation of plant cells are prepared. Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. See, for example, Weising *et al. Ann. Rev. Genet.* 22:421-477 (1988).

A DNA sequence coding for the desired *RRK* polypeptide, for example a cDNA or a genomic sequence encoding a full length protein, will be used to construct a recombinant expression cassette which can be introduced into the desired plant. An expression cassette will typically comprise the *RRK* polynucleotide operably linked to transcriptional and translational initiation regulatory sequences which will direct the

transcription of the sequence from the *RRK* gene in the intended tissues of the transformed plant.

For example, a plant promoter fragment may be employed which will direct expression of the *RRK* in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'-promoter derived from T-DNA of *Agrobacterium tumafaciens*, and other transcription initiation regions from various plant genes known to those of skill.

Alternatively, the plant promoter may direct expression of the *RRK* gene in a specific tissue or may be otherwise under more precise environmental or developmental control. Such promoters are referred to here as "inducible" promoters. Examples of environmental conditions that may effect transcription by inducible promoters include pathogen attack, anaerobic conditions, or the presence of light.

Examples of promoters under developmental control include promoters that initiate transcription only in certain tissues, such as leaves, roots, fruit, seeds, or flowers. The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

The endogenous promoters from the *RRK* genes of the invention can be used to direct expression of the genes. These promoters can also be used to direct expression of heterologous structural genes. Thus, the promoters can be used in recombinant expression cassettes to drive expression of genes conferring resistance to any number of pathogens, including fungi, bacteria, and the like.

To identify the promoters, the 5' portions of the clones described here are analyzed for sequences characteristic of promoter sequences. For instance, promoter sequence elements include the TATA box consensus sequence (TATAAT), which is usually 20 to 30 base pairs upstream of the transcription start site. In plants, further upstream from the TATA box, at positions -80 to -100, there is typically a promoter element with a series of adenines surrounding the trinucleotide G (or T) N G. J. Messing et al., in *Genetic Engineering in Plants*, pp. 221-227 (Kosage, Meredith and Hollaender, eds. 1983).

If proper polypeptide expression is desired, a polyadenylation region at the 3'-end of the *RRK* coding region should be included. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA.

The vector comprising the sequences from an *RRK* gene will typically comprise a marker gene which confers a selectable phenotype on plant cells. For example, the marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to kanamycin, G418, bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosluforon or Basta.

Such DNA constructs may be introduced into the genome of the desired plant host by a variety of conventional techniques. For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation, PEG poration, particle bombardment and microinjection of plant cell protoplasts or embryogenic callus, or the DNA constructs can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. Alternatively, the DNA constructs may be combined with suitable T-DNA flanking regions and introduced into a conventional *Agrobacterium tumefaciens* host vector. The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria.

Transformation techniques are known in the art and well described in the scientific and patent literature. The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski *et al. Embo J.* 3:2717-2722 (1984). Electroporation techniques are described in Fromm *et al. Proc. Natl. Acad. Sci. USA* 82:5824 (1985). Ballistic transformation techniques are described in Klein *et al. Nature* 327:70-73 (1987). Using a number of approaches, cereal species such as rye (de la Pena *et al.*, *Nature* 325:274-276 (1987)), corn (Rhodes *et al.*, *Science* 240:204-207 (1988)), and rice (Shimamoto *et al.*, *Nature* 338:274-276 (1989) by electroporation; Li *et al. Plant Cell Rep.* 12:250-255 (1993) by ballistic techniques) can be transformed.

Agrobacterium tumefaciens-meditated transformation techniques are well described in the scientific literature. See, for example Horsch *et al. Science* 233:496-498 (1984), and Fraley *et al. Proc. Natl. Acad. Sci. USA* 80:4803 (1983). Although *Agrobacterium* is useful primarily in dicots, certain monocots can be transformed by *Agrobacterium*. For instance, *Agrobacterium* transformation of rice is described by Hiei *et*

al, *Plant J.* 6:271-282 (1994).

Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant which possesses the transformed genotype and thus the desired *RRK*-controlled phenotype. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the *RRK* nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans *et al.*, *Protoplasts Isolation and Culture*, *Handbook of Plant Cell Culture*, pp. 124-176, MacMillilan Publishing Company, New York, 1983; and Binding, *Regeneration of Plants*, *Plant Protoplasts*, pp. 21-73, CRC Press, Boca Raton, 1985. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee *et al. Ann. Rev. of Plant Phys.* 38:467-486 (1987).

The methods of the present invention are particularly useful for incorporating the *RRK* polynucleotides into transformed plants in ways and under circumstances which are not found naturally. In particular, the *RRK* polypeptides may be expressed at times or in quantities which are not characteristic of natural plants.

One of skill will recognize that after the expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

The effect of the modification of *RRK* gene expression can be measured by detection of increases or decreases in mRNA levels using, for instance, Northern blots. In addition, the phenotypic effects of gene expression can be detected by measuring lesion length as in plants. Suitable assays for determining resistance are described in USSN 08/587,680.

The following Examples are offered by way of illustration, not limitation.

Example 1

As noted above, *Xa21* genes make up a multigene family. Pulsed field gel electrophoresis and genetic analysis have demonstrated that most of the members of the *Xa21* gene family are located in a 230 kb genomic region on chromosome 11 linked to at

least 8 major resistance genes and 1 QTL for resistance (Song, *et al.*, *Science* 270:1804 (1995); Ronald, *et al.*, *Mol. Gen. Genet.* 236:113 (1992).

This example describes six *Xa21* gene family members from the resistant rice line IRBB21, which members are designated A1, A2, C, D, E, and F. Cloning was as described in USSN 08/587,680; Song, *et al.*, *supra* and Wang, *et al.*, *Plant J.* 7, 525 (1995). DNA sequences were determined by using the Sequitherm Long Read Cycle Sequencing Kit (Epicentre Technologies) in combination with the LI-COR Model 4000L Automated Sequencer (LI-COR Inc). To fill in gaps, a primer walking strategy was performed using synthesized primers (Operon) and the Applied Biosystems 373 DNA sequencer. Genebank accession numbers are as follows: A1: U72725 (SEQ ID NO: 4); A2: U72727 (SEQ ID NO: 10); C: U72723 (SEQ ID NO: 6); D: U72726 (SEQ ID NO: 1); E: U72724 (SEQ ID NO: 8); F: U72728 (SEQ ID NO: 12); 3' flanking region of F: U72729 (SEQ ID NO: 12). The Wisconsin sequence analysis programs GAP and Pileup were used to calculate the percent identity and to carry out multiple alignments of DNA and protein sequences, respectively.

Sequence data and restriction enzyme analysis of cosmid and bacterial artificial chromosome clones indicated that the seven members are contained on 4 clones (Fig. 1). The first clone, carrying *Xa21* (described in USSN 08/587,680 and Song *et al.*, *supra*. The Genbank accession number for *Xa21* genmomic and cDNA sequences is U37133) and member C, spans a 40 kb region; the second clone includes member D, A1, and A2 and occupies a 150 kb region; clones of 40 kb and 130 kb contain members E and F, respectively. Genetic and molecular data suggests member E is inherited from the susceptible parent IR24 (P.C. Ronald, *et al.*, *Mol. Gen. Genet.* **236**, 113 (1992)).

The entire coding region, the intron, and 3' flanking region of the seven family members can be grouped into two classes. One class (designated the *Xa21* class) contains *Xa21*, as well as members D and F (SEQ ID NOs: 1 and 12). The second class (designated the A2 class) contains members A1 (SEQ ID NO:4), A2 (SEQ ID NO:10), C (SEQ ID NO:6), and E (SEQ ID NO:8). Within each class, family members share striking nucleotide sequence identity (98.0% average identity for the members of the *Xa21* class; 95.2% average identity for the members of the A2 class); compared to low levels of DNA sequence identity between members of the two classes (eg. 63.5% identity between *Xa21* and A2) (Fig. 2A). Only the *Xa21* and A1 open reading frames (ORFs) encode

receptor kinase-like proteins. The sequence of other family members contain alterations causing a premature truncation of the predicted receptor kinase-like ORF (small deletions in F and C; base pair mutations in A2; or transposon insertions in D and E). At the amino acid level, Al and XA21 share 68.6% identity overall. As shown in Figure 2B, Domains I and II, carrying the presumed signal peptide and amino terminus of the protein, are 100% identical whereas the LRR domain (domain III) of XA21 and A1 share a low level of identity (59.5%) and differ in the number of LRRs (23 vs 22 respectively). In the presumed intracellular portion, the catalytic domains (domain VIII) of XA21 and Al are highly conserved (82% identity), whereas the non-catalytic regions are divergent (64% identity for domain VII (juxtamembrane) and 38.5% identity for domain IX (carboxyl terminus)). The differences observed between members of the two classes suggest that they may differ in function. Indeed, we have found transgenic plants containing the A1 sequence are susceptible to all *Xoo* isolates tested.

A remarkable feature of the *Xa21* family members is the presence of fourteen transposable element-like sequences (M.A. Grandbastien, *et al.*, *Nature* **337:** 376 (1989); S.E.; White, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91:** 11792 (1994)). The position of these elements is shown in Fig 1. Twelve elements insert into noncoding regions; whereas two elements, named *Retrofit* and *Truncator*, integrate into the coding regions of members D and E, respectively, resulting in disruption of the ORFs of these two members (Fig. 1, number 9 and 13). *Retrofit* (SEQ ID NO:3) belongs to the *Drosophila* copia class of retrotransposons and carries a large ORF showing greatest similarity to the ORF of maize *Hopscotch* (68.6% similarity; 54.6% identity) and tobacco *Tnt1* (51.4% similarity; 31.9% identity) (M.A. Grandbastien, *et al.*, *Nature* **337:** 376 (1989); S.E.; White, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91:** 11792 (1994)). The insertion site of this element is located between the 23rd (V) and 24th (P) amino acids of the 22nd LRR creating a truncated molecule, lacking the transmembrane and kinase domains (Fig. 3A). Insertion of *Retrofit* into a presumed coding region contrasts with the observation in yeast and maize that integration of retrotransposons is biased towards noncoding regions (D.F. Voytas, *Science* **274:** 737 (1996); P. SanMiguel, *et al.*, *Science* **274:** 765 (1996)). The fact that the truncated D confers partial resistance to *Xoo* suggests that transposition events at the *Xa21* locus can alter expression of resistance.

Truncator, 2913 bp, represents a novel transposon-like sequence carrying 9

bp terminal inverted repeats (TIRs). The sequence shows no significant homology to any sequence in the database and contains no obvious ORFs. Interestingly, insertion of this element into the amino terminus of the kinase domain of member E would presumably result in premature truncation of the receptor kinase resulting in a receptor-like molecule structurally similar to the tomato fungal resistance gene products Cf9 and Cf2 (Fig. 3B) (D.A. Jones, *et al.*, *Science*, **266**: 789 (1994); M.S. Dixon, *et al.*, *Cell* 84:451 (1996)).

In addition to the transposition events presented above, recombination between different family members was also found to play an important role in the evolution of the *Xa21* locus. A 269 bp highly conserved (HC) region, located immediately downstream of the start codon of all seven family members marks the site of intragenic recombination events (Fig. 2A). The HC region, has a high G/C content (61.8% for *Xa21*) hallmarked by the typical G/C rich restriction enzyme recognition site Not I. At the amino acid level, the HC region spans domain I and domain H of XA21 and shares nearly 100% identity among seven family members.

The HC region delimits four classes of DNA sequences (~1.3 kb) upstream of the HC region. The 5' flanking region of family member F is divergent from that of other family members (less than 40% identity). The precise breakpoint (from sequence similarity to divergence) between *Xa21* and F is located within the HC region, 120 bp downstream from the start codon. This sudden change of sequence identity is unlikely due to random events such as transposon insertion or deletion because such events would presumably lead to an altered coding region. This is not the case; the deduced amino acid sequence of F maintains the receptor kinase like ORF. These results suggest that a recombination event occurred in the HC region resulting in the formation of a chimeric sequence containing the 5' flanking region of F and a downstream region (including coding region, intron, and 3' flanking region) of the *Xa21* class.

In further support of the idea that the HC region mediates intragenic recombination, we also observed apparent recombination breakpoints near or within the HC region for gene family members E, A1, and C. For E, the 5' flanking region is divergent from all other members whereas the 3' downstream regions belong to the A2 class. The sudden change of DNA identity can be explained by a recombination event between a progenitor A2-type gene and an unknown family member. The likely recombination breakpoint in E is located 105 bp upstream of the HC region since

sequences upstream of this site are quite different, compared with a high level of DNA sequence identity downstream of this site.

The nearly identical DNA sequences of C and A1 provide the most striking example of an HC mediated recombination event. For example, the 5' flanking region of C shows nearly perfect identity (99.2%) to that of *Xa21*, whereas the downstream region of C belongs to the A2 class. The high level of identity between the 5' flanking sequences of *Xa21* and C extends 3.8 kb upstream. This upstream region includes the functional promoter for the *Xa21* gene (W.-Y. Song, *et al.*, *Science* **270**:1804 (1995)). These results strongly suggest that C was created by a recombination event in the HC region between progenitors of the *Xa21* and A2 classes. The likely recombination breakpoint in member C is delimited by two characteristic deletions: one is located at position -37 and is only present in *Xa21* class members *(Xa21*, D, C, and A1); another deletion is located at position 255 and occurs in all A2 class members.

From these results it is clear that we have identified a highly conserved, G/C rich region in the gene family and that this region appears to be involved in high frequency recombination between family members. Not only is the HC region present in *O. longistaminata,* but is also present in *Xa21* family members of the cultivated rice species *O. sativa* (The clone RG103, spanning the HC region of an *Xa21* gene family member was isolated from *0. sativa* cultivar IR36 (3, S. Mcouch, *et al.*, *Theoret. Appl. Genet.* **76**:815 (1988)). Genebank accession number of RG103 is U82168. The mechanism for HC region-mediated recombination is unknown; however, two models can be envisioned. First, this region may mediate programmed recombination similar to that observed in African trypanosomes (R.H.A. Plasterk, *Trends Genet* **8**, 403 (1992)). In trypanosomes, antigenic variation is controlled by a variant surface glycoprotein (VSG), which is encoded by a member of a multigene family containing more than 1000 members. Recombination at stretches of highly conserved nucleotides between silent and expressed members of the VSG gene family leads to expression of new antigens. Alternatively, HC mediated recombination may be an example of an ectopic recombination event where the HC region serves as a recombination initiation site (T.D. Petes, *et al.*, *Annu. Rev. Genet.* **22**:147 (1988); A. Nicolas, *et al.*, *Nature* **338**: 35 (1989)). Frequent recombination in this region would maintain the conservation of the HC region but allow flanking sequences to diverge. Over time, mismatch repair would lead to homogenization of the HC region and

result in an overall increased G/C content as has been observed in yeast (Brown T., *et al.*, *Cell* 54, 705 (1988)).

Evidence for recombination in intergenic regions of the *Xa21* family members was also observed. First, sequences in the 5' flanking region of members C and *Xa21* are identical for 3.8 kb and then abruptly diverge. Interestingly, the same site of divergence is observed in the 3' flanking regions of *Xa21* and member F (Fig. 4). The presence of a conserved site of divergence suggests not only that this is a recombination breakpoint but that the *Xa21*/C cluster and member F are generated from the same progenitor. Second, the sequence of a 14742 bp region spanning the *Xa21*/C cluster shows 97.7% identity to the corresponding sequence (14871 bp) of the D/A1/A2 cluster (Fig. 1), suggesting these regions evolved through sequence duplication. This duplication process can be explained by a presumed unequal cross-over event in the intergenic region of these two clusters.

Example 2

Using PCR amplification techniques as described in USSN 08/587,680, Xa21 genes were isolated from cassava (SEQ ID NOS: 13-14), maize (SEQ ID NO: 15-16) and tomato (SEQ ID. NOs: 17-29). The following is a description of the methods used to isolate TRK1-7 from tomato. The same general procedure was used for maize and cassava.

We designed primers in conserved regions of both the Leucine Rich Repeat (LRR) region and the serine-threonine kinase domain of Xa21. The PCR products should amplify between these two domains and therefore span the transmembrane domain. So far, two sets of primers have proven successful to amplify three homologues of Xa21 in tomato.

The first clone TRK1 is a cDNA and the encoded polypeptide (SEQ ID NOs:17and 18). This clone is present as one or two copies in the tomato genome and one copy maps to the short arm of chromosome 1 in the proximity of a resistance gene to Xanthomonas campestris pv. vesicatoria (Rx1)(Zu *et al.* (1995) *Genetics* 41:675-682).

The second clone TRK2 (SEQ ID NO:19) is a 496bp PCR product with an ORF encoding a polypeptide (SEQ ID NO:20). TRK2 maps within a few cM of mcn (figure 4) a mutation on chromosome 3 that mimics disease lesions. A third clone TRK3

(SEQ ID Nos: 21 and 22) is a 473bp fragment and maps to chromosome 8 near an erecta like mutant. TRK4-7 (SEQ ID Nos: 23-29) are further PCR products and encoded polypeptides

Primers that have been proven useful are as follows.

1. LRR region

L3a. TCA AGC AAC AAT TTG TCA GGN CA(A/G) AT(A/C/T) CC

2. Kinase region

K1a CGC CTT AGG ATT TTC AAG CTT TCC (T/C)TT (G/A)TA NAC

K2a. TAA CAG CAC ATT GCT TGA TTT NAN (G/A)TC NCG (G/A)TG

K2b. TAA CAG CAC ATT GCT TGA TTT NAN (G/A)TC (G/A)CA (G/A)TG

K2c. TAA CAG CAC ATT GCT TGA TTT NAN (G/A)TC (T/C)CT (G/A)TG

The following combinations of primers are preferred:

L3a+K1a then L3u+K1u

L3a+K2a then L3u+K2u

L3a+K2b then L3u+K2u or

L3a+K2c then L3u+K2u.

PCR conditions

first cycle

94 for 30 s

55 for 30 s

72 for 1 min

For the next 19 cycles, the annealing temperature drops 1degree C every cycle. After 20 cyles, 10 min at 72. After inital amplification as second round of amplification is performed with the following specific primers with 1 microliter of the previous PCR.

L3u. TCA AGC AAC AAT TTG TCA

K1u. CGC CTT AGG ATT TTC AAG CTT

K2u. TAA CAG CAC ATT GCT TGA

The conditions for this amplification are:

35 cycles

94 15 sec

55 15 s

72 1 mn

after 35 cyles, 72 for 10 min

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid construct comprising an *RRK* polynucleotide sequence, which polynucleotide hybridizes to SEQ ID NOs: 1, 4, 6, 8, 10, or 11 under stringent conditions.

2. The nucleic acid construct of claim 1, wherein the *RRK* polynucleotide sequence encodes an *RRK* polypeptide having an leucine rich repeat motif.

3. The nucleic acid construct of claim 1, wherein the *RRK* polynucleotide sequence encodes an *RRK* polypeptide having a cytoplasmic protein kinase domain.

4. The nucleic acid construct of claim 1, wherein the polynucleotide sequence is a full length gene.

5. The nucleic acid construct of claim 1, wherein the *Xa21* polynucleotide is as shown in SEQ ID NOs: 1, 4, 6, 8, 10, or 11.

6. The nucleic acid construct of claim 1, further comprising a promoter operably linked to the *RRK* polynucleotide sequence.

7. The nucleic acid construct of claim 1, wherein the promoter is a tissue-specific promoter.

8. The nucleic acid construct of claim 1, wherein the promoter is a constitutive promoter.

9. An isolated nucleic acid construct comprising a cassava *RRK* polynucleotide sequence, which polynucleotide hybridizes to SEQ ID NO: 13 under stringent conditions.

10. The isolated nucleic acid construct of claim 9, which is SEQ ID NO:

13.

11. An isolated nucleic acid construct comprising a maize *RRK* polynucleotide sequence, which polynucleotide hybridizes to SEQ ID NOs: 15 or 16 under stringent conditions.

12. The isolated nucleic acid construct of claim 11, which is SEQ ID NO: 15 or SEQ ID NO: 16.

13. An isolated nucleic acid construct comprising a tomato *RRK* polynucleotide sequence, which polynucleotide hybridizes to SEQ ID NOs: 17, 19, or 21 under stringent conditions.

14. The isolated nucleic acid construct of claim 13, which is SEQ ID NO: 17, SEQ ID NO:19, or SEQ ID NO:21.

15. A transgenic plant comprising a recombinant expression cassette comprising a plant promoter operably linked to a *Xa21* polynucleotide sequence of claim 1.

16. A method of enhancing resistance to *Xanthomonas* in a plant, the method comprising introducing into the plant a recombinant expression cassette comprising a plant promoter operably linked to an *RRK* polynucleotide sequence of claim 1.

17. The method of claim 16, wherein the plant tissue is from rice.

18. The method of claim 16, wherein the plant tissue is from tomato.

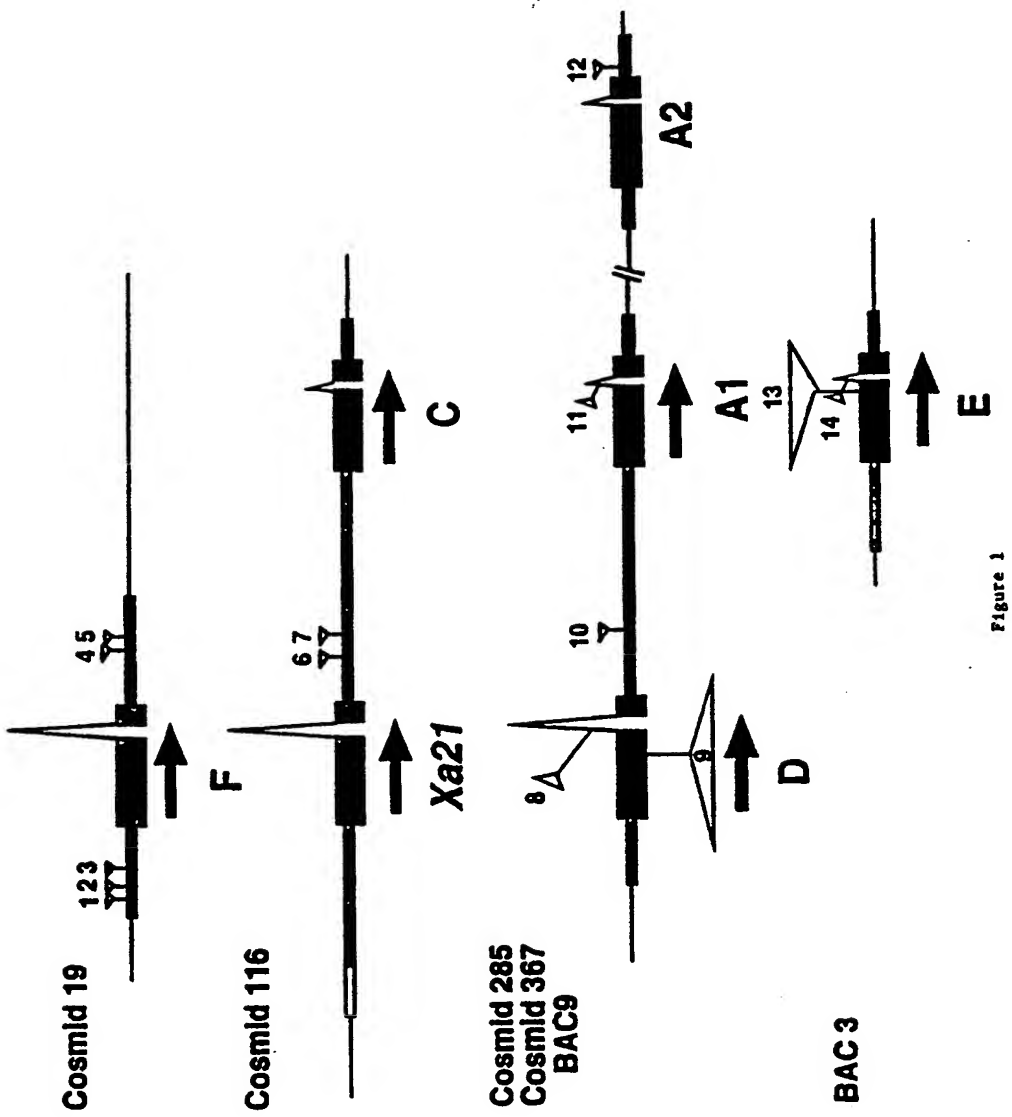


Figure 1

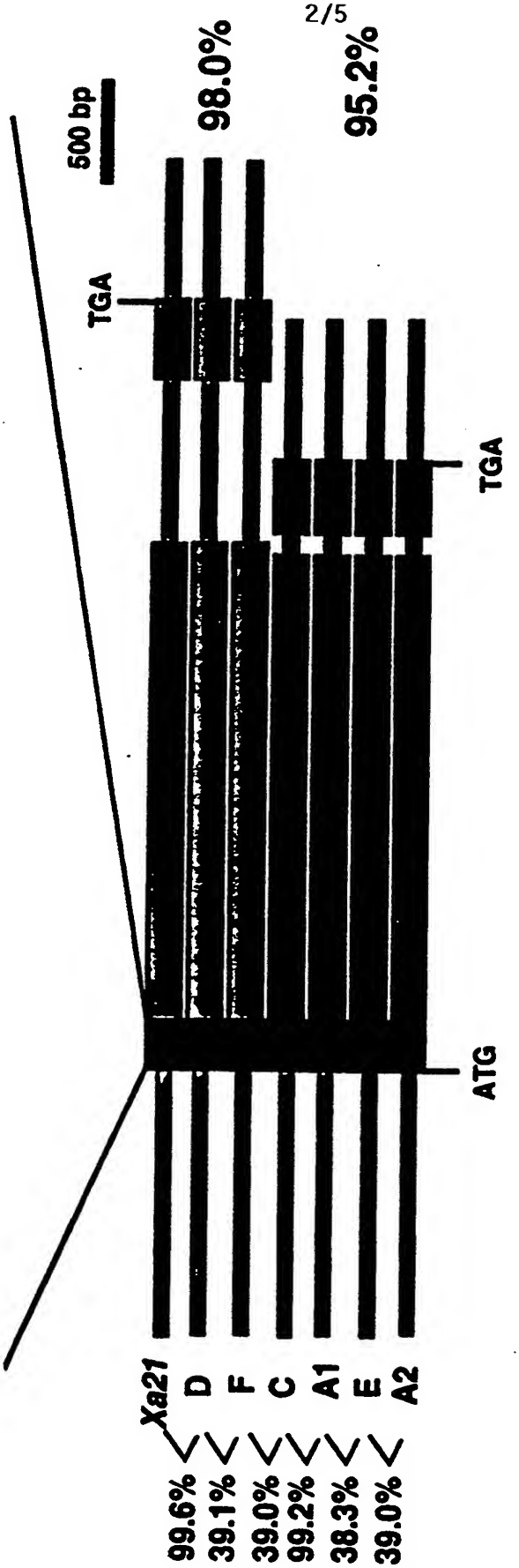
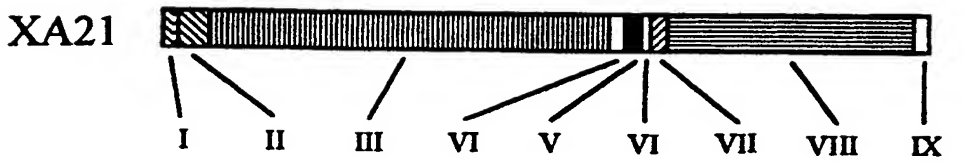


Figure 2A



A1(%)	100	100	59.5	57.1	34.6	83.3	64.0	82.0	38.5

Figure 2B

FIGURE 3A

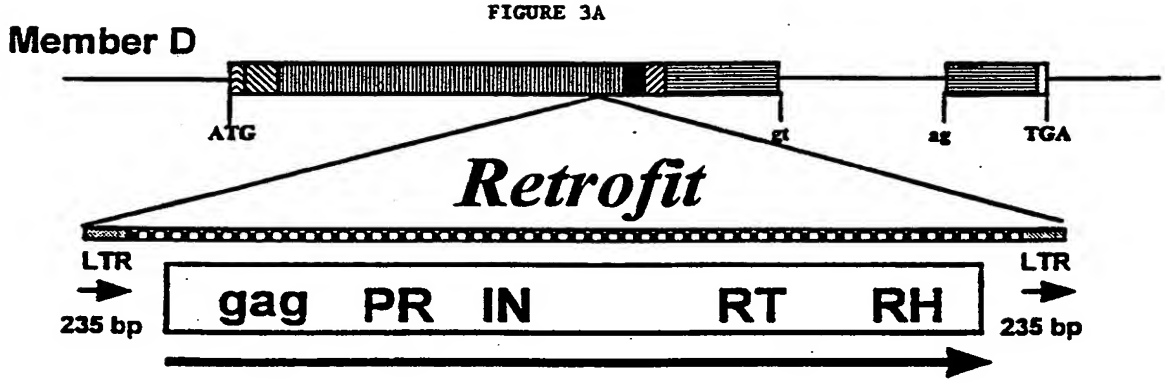
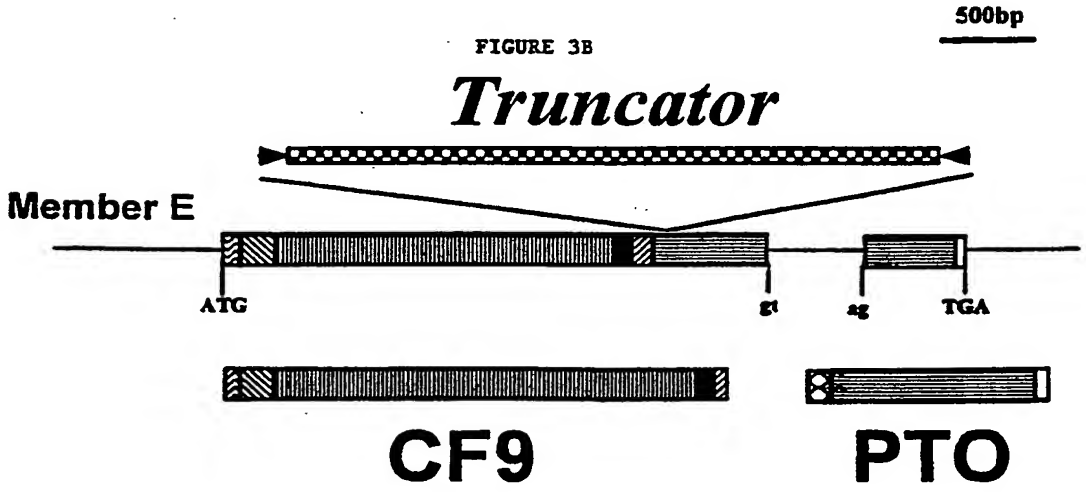


FIGURE 3B



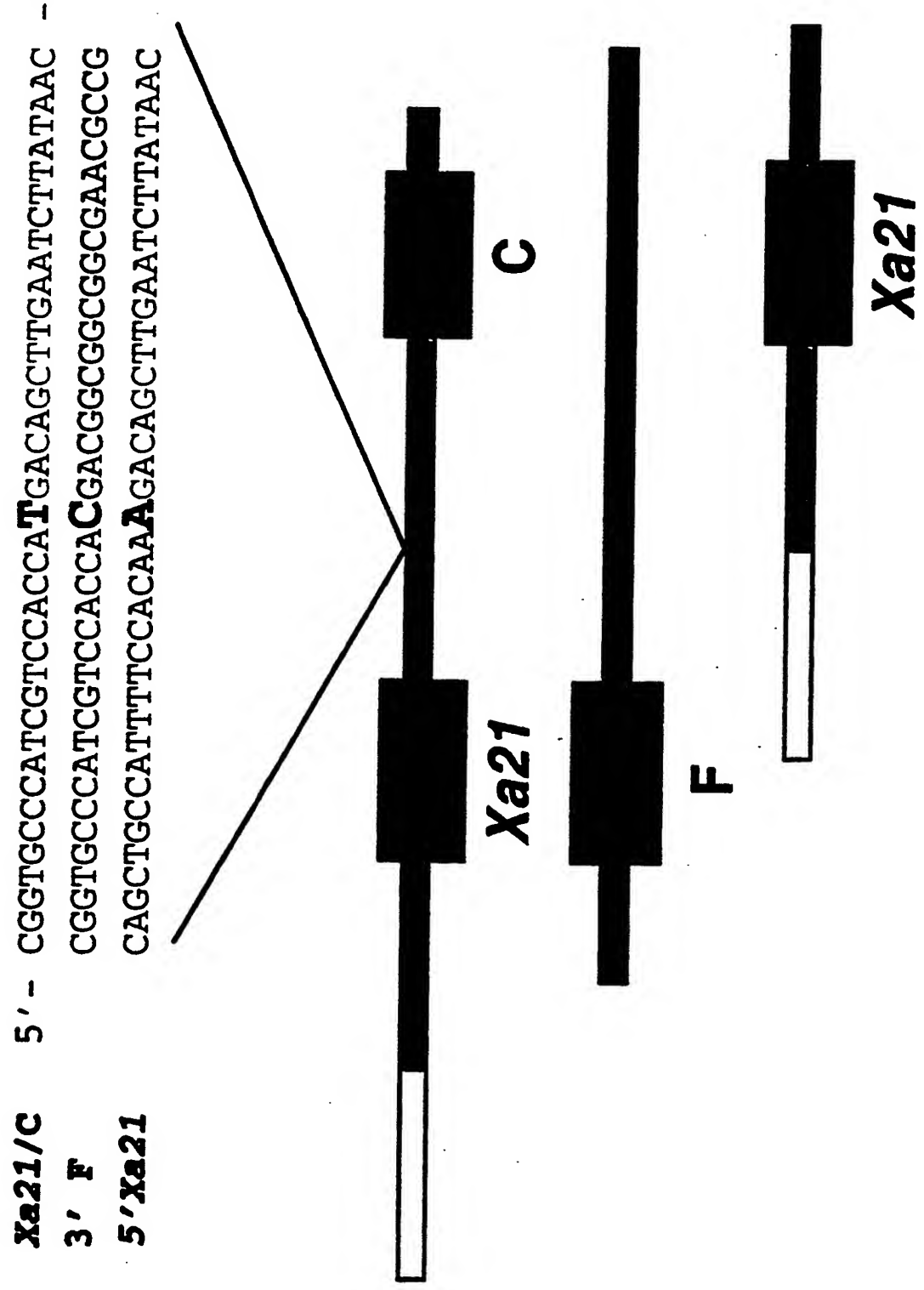


Figure 4

Sequence Listing

SEQ ID NO:1

```
DEFINITION  Oryza longistaminata receptor-like protein, family member D, and
            retrofit (gag/pol) genes, complete cds.
ACCESSION   U72726
SOURCE      long-staminate rice.
ORGANISM    Oryza longistaminata
            Eukaryotae; mitochondrial eukaryotes; Viridiplantae;
            Charophyta/Embryophyta group; Embryophyta; Magnoliophyta;
            Liliopsida; Poales; Poaceae; Oryza.
COMMENT     U72725 and U72726 are separated by a large AT rich
microsatellite region.
FEATURES             Location/Qualifiers
     source          1..13341
                     /organism="Oryza longistaminata"
                     /strain="IRBB21"
                     /chromosome="11"
                     /map="11q, RG103"
     CDS             2367..4205
                     /note="Xa21 gene family member D
                     /codon_start=1
                     /product="receptor kinase-like protein"

    misc_feature     4201..9071
                     /note="retrofit, a copia-like, transposon-like element"
     gene            4484..8821
                     /gene="gag/pol"
     CDS             4484..8821
                     /gene="gag/pol"
                     /codon_start=1
                     /product="retrofit"

     intron          9915..11712
     misc_feature    10020..10975
                     /note="Krispie, transposon-like element"
     3' flanking     12114..13341
     misc_feature    12626..12750
                     /note="Pop-012, transposon-like element"
                     13040..13248
                     /note="Ds-rice2, transposon-like element"
```

```
   1 aagcttcatt ggtttcttca gttatactta cgtaggtttt tcctgtatac ataaatacgt
  61 aacagagtaa gggaattaga ttgtttaaaa taaaatacat ataatctaat agcctaaaat
 121 atcaggtcca ctgacagtgg cggatctagg atttagaata tgggtggtcc gacctaattt
 181 tttcctaaac atactaaatc taacgatggt aatatatact atgcaagtat agataataga
 241 atagaccaaa agtgtatcat gctatattaa taaagcatct taaaacatat ataattaata
 301 attacctaaa attttgactt aaagaagctc acatggctat aaaagtttaa agaaaattac
 361 catactaatt tttcttctta tcgggtctac gccttctaat ggccatgaaa gtggtcgtta
 421 tatcttcttc cttcactctt aagaaaacat cccgcttaat ggatgtgtct atactatcat
 481 ccaaaagctc atcacccatc ttttttctca accatcatta gtaaatgcat cagttctact
 541 ataatttaat atcacaatgc acaggagtaa agagttcaaa atttcaaaac tgaaaattga
 601 aaaaaaagta aaaaaaaaat agaaaacctt tttgttttgg cttggtgcag gtctgcacca
 661 gtgcgctagt gcggcactgc ggcggcagcg gccaaggtgt cgacgcgcgt gcgtggcccg
 721 gtggcgctcg ctctcacgat ctgatcagat cgctgatcgc gtcggcgtcg cgactcgcga
 781 gggcgaggag gagagcgaca gagagtcctg cgacggcgcg acgcttcggt ttcttaattc
 841 cgaacgatta gatacaccgt acacgcgcgt gtggtgtggg gcctgtggta atctaatggt
```

```
 901 ttaaaatatt gggtccacca atttaagtga aaatcgacgg ttagatatga tagagctacg
 961 tggcagccta agagcgtttg taggagtccc acgtggcggt ttgagagcgt ttgtaggaag
1021 tttaatggac ttttagtata taatagatat ttataatttt attaagtacc ctaattttcc
1081 ctaaacaatt tttctctctc atcgtatttc catatatctt tttgagataa taatggatat
1141 aaacatagct agaaatgtaa atgttcacct tgcatcaata ggggatgaag ttgctaacct
1201 tttagatctc ctcgatttgt ataatataac caaaatattt tcaccaaaaa tttcgttaaa
1261 catccgagat atttgttgtt tttgccgatc gagcaaagat tagtagtcca gcagtgtctg
1321 caccaccacc atcgtgataa tgcatcttgt gtgttattct tgatgagaaa atacgtagtg
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1561 cgatccaacg atagcttcca agtttccact acatatgtgg tttgcactat atattttccc
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1741 tttctttaaa aaatatatta atctattttt taagtttaaa ataattacta cttaattaat
1801 tatacactaa cagcttattt cgttctacgt atcttgtcaa ttttcgctat tccttttcttc
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1981 ttccatgccg aagtgccgac gatgagaccg tgttcgatgc atctttgtat ggcatctagg
2041 gacaaagagc atagagtccc taccatagta cctgctcgcg tagaagactt gacgagaaga
2101 ccgactgcta caccttggtg tgtaataata tcgtgttgtg tgtaccatgc atactccttt
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SEQ ID NO:4

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DEFINITION  Oryza longistaminata receptor kinase-like protein gene, family
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ACCESSION   U72725
SOURCE      long-staminate rice.
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COMMENT     U72725 and U72726 are separated by a large AT rich
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FEATURES             Location/Qualifiers
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DEFINITION Oryza longistaminata receptor kinase-like protein (Xa21) gene, complete cds and family member C, pseudogene.
ACCESSION U72723
SOURCE long-staminate rice.
ORGANISM Oryza longistaminata
Eukaryotae; mitochondrial eukaryotes; Viridiplantae; Charophyta/Embryophyta group; Embryophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Oryza.

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SOURCE rice.
ORGANISM Oryza sativa
Eukaryotae; mitochondrial eukaryotes; Viridiplantae; Charophyta/Embryophyta group; Embryophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Oryza.

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SEQ ID NO:10

DEFINITION Oryza longistaminata receptor kinase-like protein, family member A2, pseudogene sequence.
ACCESSION U72727
SOURCE long-staminate rice.
ORGANISM Oryza longistaminata
Eukaryotae; mitochondrial eukaryotes; Viridiplantae; Charophyta/Embryophyta group; Embryophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Oryza.

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SEQ ID NO:11

ORGANISM Oryza longistaminata
Eukaryotae; mitochondrial eukaryotes; Viridiplantae;
Charophyta/Embryophyta group; Embryophyta; Magnoliophyta;
Liliopsida; Poales; Poaceae; Oryza.

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KYFRNNGTSECMSAIAGSYGYIAPEYAYTLKIDEKSDVYSFGVVLLELITGR
RPVGNFGEEGMDIVQWAKTETKWSKEGVVKILDERLKNVAIVEAMQVFFVAM
LCVEEYSIERPTMREVVQMLSQAKQPNTFQIQ

SNCGSTYCMLGNTPFVSFAKVKSQINLSEVVAKLICNYVRS.GMISNYILS
TWNSVLDV.N.Y.YPRDVWRISKESR???LIP??????R?R?PPILTDSRSRRHQ

SEQ ID NO: 19

TRK2

TGACTCTCTCTGTCTCTCTCTGTTCGCAGCCCCAAAAAGTAGGGTTAGGG
CTAGGGTTTTTGAGTTTCAAAACCCCATTTCTGGTTCCTATAATCTTCAC
ATACAAGGGGAGTTTGTCTCTGTTGCATTCTTTGAAGACCCTTTTGGGGT
TTTACTAATGGGTCGTTGTTGTTTTGTCATCAAATGGTACTATCATGACA
TACCCTTGAAAGTTTTTCTCATCCTTTGTGTTTTCTTCTTAGTTCATGGC
TATGCACTTTCCTCGGATTCGGATAAATCAGCGCTCTTGGAGTTAAAGGC
CTCATTTTCAGATTCCTCTGGAGTGATTTCTAGCTGGAGCTCCAGAAATA
ATGATCACTGTTCATGGTTTGGTGTCTCCTGTGATTCCGATTCACGTGTT
GTGGCTTTGAACATCACTGGAGGTAATTTGGGTTCTTTATCTTGTGCTAA
AATTGCTCAATTTCCTTTGTATGGCTTTGGAATTACAAGGGTTTGTGCTA
ATAATAGTGTCAAGCTTGTTGGTAAAGTACCTCTCGCAATATCAAAATTA
ACTGAACTAAGGGTTTTATCCTTGCCTTTTAATGAATTGCGTGGTGATAT
TCCATTGGGAATTTGGGATATGGACAAACTTGAAGTTTTGGATCTGCAAG
GGAATTTAATTACTGGGTCTTTGCCATTGGAGTTTAAGGGGTTGAGGAAA
TTGAGGGTTTTAAACTTGGGTTTTAATCAGATTGTGGGTGCCATACCGAA
TTCCTTGTCAAATTGCCTTGCTCTACAAATCTTTAATCTTGCTGGAAATA
GGGTAAATGGGACCATTCCAGCATTCATTGGTGGATTTGAAGATCTGAGG
GGAATCTACCTGTCTTTTAATGAGCTTAGCGGGTCTATTCCTGGTGAAAT
TGGGCGTTCTTGTGAGAAGCTTCAAAGTCTAGAGATGGCAGGTAATATCT
TAGGTGGTGTTATTCCAAAAAGTTTAGGGAACTGCACACGGTTGCAGTCA
CTTGTCTTATATTCAAATTTGTTGGAAGAGGCTATTCCAGCTGAATTTGG
TCAACTAACTGAGCTCGAGATTCTTGATTTGTCTAGGAACAGCCTAAGTG
GTCGACTACCATCTGAGCTGGGAAACTGCTCGAAACTATCCATTCTTGTA
CTGTCAAGTTTGTGGGATCCCCTTCCAAATGTGTCTGATTCAGCTCATAC
TACTGATGAGTTTAACTTTTTTGAAGGCACAATCCCATCAGAGATCACCA
GGCTTCCTAGTTTGAGAATGATATGGGCTCCCAGGTCAACTCTTTCAGGA
AAATTTCCTGGCAGTTGGGGTGCTTGTGACAATTTGGAGATCGTGAACTT
GGCTCAAAATTATTATACTGGAGTGATTCCTGAGGAATTGGGTAGCTGCC
AGAAGTTGCATTTTCTTGACTTGAGCTCAAATAGGCTGACTGGACAGCTT
GTTGAGAAACTGCCAGTCCCTTGCATGTTTGTGTTCGATGTGAGTGGGAA
TTATCTCTCTGGTTCAATTCCCAGGTTTTCCAATTACAGTTGTGCTCATG
TTGTTTCCAGCGGTGGAGAGCCATTTGGGCCCTATGATACATCATCTGCA
TATCTAGCACATTTCACCAGTAGAAGTGTTCTAGACACTACATTATTTGC
AGGTGATGGTAACCATGCAGTATTTCATAATTTCGGTGTTAACAACTTCA
CGGGAAATTTACCGCCTTCCATGCTAATTGCACCTGAAATGTTAGGCAAA
CAAATTGTATACGCATTTCTTGCTGGTAGTAACAGGTTTACTGGACCTTT
TGCTGGTAACTTGTTCGAGAAATGTCATGAATTGAATGGAATGATTGTTA
ATGTAAGCAATAATGCGTTGTCAGGTCAAATCCCAGAGGATATTGGTGCA
ATTTGTGGGTCTCTTAGGCTGTTGGATGGATCCAAAAATCAGATTGTTGG
GACAGTCCCTCCGAGTTTAGGGAGTCTGGTTTCATTAGTTGCTCTCAATT
TAAGTTGGAACCACCTGCGAGGTCAGATTCCTAGCAGACTTGGCCAGATA
AAGGATCTCAGTTACCTCTCTTTGGCTGGCAATAATCTGGTTGGCCCAAT
CCCCTCAAGTTTTGGCCAATTGCACTCTTTAGAAACGCTTGAACTTTCTT
CGAATTCTTTGTCTGGTGAAATTCCAAATAATCTGGTAAATTTGAGGAAT
TTGACTTCCCTTCTTCTGAACAACAACAATTTATCAGGGAAAATACCTTC
AGGCTTGGCCAATGTGACCACACTGGCAGCATTTAACGTTTCTTTCAATA
ATCTGTCTGGGCCACTGCCTCTTAACAAAGATTTGATGAAGTGTAATAGT
GTTCAGGGAAACCCCTTTCTGCAATCGTGCCATGTATTTTCTCTATCAAC
ACCTTCTACAGATCAGCAGGGAAGAATAGGGGACTCACAAGATTCTGCTG
CGTCTCCTTCAGGTTCAACCCAGAAAGGAGGGAGCAGCGGTTTCAACTCC
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TGCTCTGATAGTCCTGTTCTTTTACACCAGAAAATGGAATCCAAGATCTA
GAGTTGCTGGATCTACCAGGAAAGAAGTCACAGTGTTTACAGAAGTTCCG

GTTCCTTTAACATTTGAAAATGTAGTGCGGGCCACAGGGAGCTTCAATGC
AAGCAATTGCATAGGCAGTGGAGGTTTTGGAGCAACATACAAAGCGGAGA
TTGCACCAGGGTTCCTAGTGGCAGTAAAGCGACTTGCTGTAGGACGTTTT
CAGGGGATTCAACAGTTTGATGCAGAAATCAGAACTCTGGGGAGGCTTCG
ACATCCAAACCTCGTAACTCTGATAGGATATCATAATAGTGAAACAGAAA
TGTTTCTGATCTATAACTATTTGCCAGGTGGTAATTTGGAAAAGTTTATT
CAGGAGAGGTCTACAAGGGCTGTGGACTGGAGGGTTCTTCACAAGATTGC
TTTGGATGTAGCCCGTGCACTTGCTTACCTGCATGATCAGTGTGTACCAC
GTGTGCTTCATCGTGATGTGAAGCCGAGCAACATTTTATTGGATGAGGAG
TATAATGCATATTTATCTGATTTTGGTTTGGCTAGATTACTGGGAACTTC
AGAGACCCATGCAACTACTGGTGTGGCGGGAACTTTTGGATATGTTGCTC
CTGAATATGCCATGACTTGCCGCGTCTCGGACAAGGCTGATGTCTACAGT
TATGGGGTTGTGTTGCTTGAGTTAATATCAGATAAGAAAGCACTAGATCC
GTCTTTCTCTTCTTATGGAAATGGATTCAATATTGTAGCTTGGGCATGCA
TGCTTTTACGCAGGGCCGTGCTAAGGAGTTCTTTACGGCTGGTCTATGGG
ATTCAGGTCCACATGATGATTTGGATGAGGTCCTACACTTGGCAGTGGTC
TGCACGGTTGACTCTCTTTCTACTAGACCAACAATGAAGCAAGTAGTAAG
ACGGTTGAAGCAACTTCAACCCCCGTCGTGTTAGCTGCGGCATGTGTTTT
GGATAGGATATGGTTTAGCCCAATTGTAATNTTAAAACTTGCCCTTGATA
GTAAGGTGTATTTGGGTGTCTCGTATTAGGTTCAGATTTGTATTTGTAGC
CTGCTTGTGAATTGTAGTATATAGCCAGCCCCC:ATTTTTCC:ATGTCAT
GTCCC:TAATTAGGGGGTGTGCAGATTCTTCT:GCAGAAGAGTGCAGATA
CTTGTCTTCAACATGTACC:ACATTTTTTTTTGTTTGTTAAATAAGAGCA
AAAAATAGGAACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA:::GC

SEQ ID NO: 20
TRK2

DSLCLSLFAAPKSRVRARVFEFQNPISGSYNLHIQGEFVSVAFFEDPFGVLLMGR
CCFVIKWYYHDIPLKVFLILCVFFLVHGYALSSDSDKSALLELKASFSDSSGVIS
SWSSRNNDHCSWFGVSCDSDS
RVVALNITGGNLGSLSCAKIAQFPLYGFG
ITRVCANNSV
KLVGKVPLA
ISKLTELRVLSLPF NELRGDIPLG
IWDMDKLEVLDLQG NLITGSLPLE
FKGLRKLRVLNLGF NQIVGAIPNS
LSNCLALQIFNLAG NRVNGTIPAF
IGGFEDLRGIYLSF NELSGSIPGE
IGRSCEKLQSLEMAGNILGGVIPKS
LGNCTRLQSLVLYS NLLEEAIPAE
FGQLTELEILDLSR NSLSGRLPSE
LGNCSKLSILVLSSLWDP
LPNVSDSAHTTDEF NFFEGTIPSE
ITRLPSFENDMAPR STLSGKFPGS
WGACDNLEIVNLAQ NYYTGVIPEE
LGSCQKLHFLDLSS NRLTGQLVEK
LPVPCMFVFDVSG NYLSGSIPRF
SNYSCAHVVSSGGEPFGPYDTSSAYLAHFTSRSVLDTTLF
AGDGNHAVFHNFGV NNFTGNLPPS
MLIAPEMLGKQIVYAFLAGSNRFTGPFAGNL
FEKCHELNGMIVNVSNNALSGQIPED
IGAICGSLRLLDGSKNQIVGTVPPS
LGSLVSLVALNLSW NHLRGQIPSR
LGQIKDLSYLSLAG NNLVGPIPSS
FGQLHSLETLELSS NSLSGEIPNN
LVNLRNLTSLLLNN NNLSGKIPSG
LANVTTLAAFNVSF NNLSGPLPLN

KDLMKCNSVQGNPFLQSCHVFSLSTPSTDQQGRIGDSQDSAASPSGSTQKGGSSGFNSIEIASITSAAAIVSVLLA
LIVLFFYT
RKWNPRSRVAGSTRKEVTVFTEVPVPLTFE
NVVRATGSFNASNCIGSGGFGATYKAEIAPGFLVAVKRLAVGRFQGIQQFDAEIRTLG
RLRHPNLVTLIGYHNSETEMFLIYNYLPGGNLEKFIQERSTRAVDWRVLHKIALDVAR
ALAYLHDQCVPRVLHRDVKPSNILLDEEYNAYLSDFGLARLLGT
SETHATTGVAGTFGYVAPEYAMTCRVSDKADVYSYGVVLLELIS
DKKALDPSFSSYGNGFNIVAWACMLLRRAVLRSSLRLVYGIQVH
MMIWMRSYTWQWSARLTLFLLDQQ
.SK..DG.SNFNPRRVSCGMCFG.DMV.PNCN?KTCP...GVFGCLVLGSDLYL.PACE
L.YIASP?FF?CHV?.LGGVQIL?AEECRYLSSTCT?IFFCLLNKSKK.EPKKKKKKKKK??

SEQ ID NO: 21
TRK3
TCAAGCAACAATTTGTCAGGCCAGATTCCCAAGTCCTTGAGGAATCTTGAACATCTCATGTATTTCAATGTCTCGT
TCAATGGGCTCATGGGTGAAATTCCAGATGGAGGGCCATTCGTAAATTTTACAGCTGAATCATTCATGGGTAACCC
TGCATTATGTGGATCATCACGCTTCCGTGTGATGCAATGCAGAGTCACTAGTCTTGAAAGAAAAGGAAAGAGTAGA
GTCTTAACTTCTGTTCTTGCATCAGCTTCCTCAGGAGTTGTAGTCACGACCATTTTCATCATTTGGTTTCTGAAAT
GCCGAAAAAGGAGTACGGAACTTCCTCTAGTTGATACATTTGGTCAGGTACATAAGAGGATTTCGTACTATGATAT
TCCTCAAGGGACAAACAGCTTTGATGAAGCAAACTTGATTGGAAGGGGGAGCCTTGGTTTGGTCTACAAAGGAAAG
CTTGAAAATCCTAAGCG

SEQ ID NO: 22
TRK3

SSNNLSGQIPKSLRNLEHLMYFNVSFNGLMGEIPDGGPFVNFTAESFMGNPALCGSSRFRVMQCRVTSLERKGKSR
VLTSVLASASSGVVVTTIFIIWFLKCRKRSTELPLVDTFGQVHKRISYYDIPQGTNSFDEANLIGRGSLLGLVYKG
KLENPKR

SEQ ID NO:23
TRK4
AGCTTGGATCAATTTAACAATGATAAACTGCAATTTAGCTGGTCCTTTGCCTGAATTTCTTGGAACTATGTCTTCT
TTAGAGGTTTTGTTGTTGTCTACAAATAGGCTTTCAGGGCCTATTCCAGGTACTTTCAAGGATGCAGTGCTGAAGA
TGCTTTGGTTGAACGATCAGTCGGGTGATGGTATGAGTGGTTCAATAGATGTTGTTGCAACTATGGTATCACTTAC
ACATCTTTGGCTTCATGGGAATCAATTTTCAGGTAAAATCCCAGTAGAGATTGGTAATCTAACAAATCTGAAGGAT
CTCAGTGTGAATACAAATAACCTTGTTGGATTAATCCCTGAAAGTTTAGCTAATATGCCATTAGACAATCTTGATT
TGAATAATAATCATTTTATGGGACCAGTTCCTAAGTTCAAGGCTACTAATGTTAGTTTTATGTCCAACTCTTTTTG
TCAAACCAAACAAGGAGCAGTATGTGCCCCTGAGGTTATGGCACTTTTAGAGTTTCTTGATGGGGTGAATTATCCT
TCTAGGCTTGTTGAATCATGGTCTGGAAACAACCCTTGTGACGGACGTTGGTGGGGAATAAGCTGTGACGATAACC
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CGTTACTCACATTTATCTTGAATCAAATAATCTTTCTGGTTTTGTTCCATCTAGTTGGACTAGTTTGAAATCTCTG
TCTATTCTTGATTTGAGTAATAACAATATTTCCCCACCTTTGCCTAAATTTACCACCCCTTTGAAACTTGTTCTAA
ATGGAAATCCAAAGCTGACTTCTAATCCTCCTGGAGCAAATCCTTCACCAAACAACAGCACAACTCCTGCAGATTC
ACCCACGTCGTCTGTACCATCTTCACGACCCAACAGTTCAAGCTCTGTGATCTTTAAACCCAGTGAACAGTCACCC
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CTATTCCACTGTACATTTATGTCTGTAAGAAGAGTAAAGATAAGCATCAAGCTCCAACTGCTCTTGTGGTTCATCC
TAGAGATCCGTCTGATTCGGATAATGTAGTCAAGATTGCGATTGCCAATCAGACTAATGGAAGTCTTTCCACAGTA
AATGCAAGTGGTTCTGCTAGCATACACAGTGGTGAATCCCATTTGATCGAAGCTGGGAATTTGCTCATATCGGTTC
AAGTACTTCGGAATGTGACTAAGAATTTTTCTCCGGAAAATGAACTTGGACGTGGTGGTTTTGGTGTGGTTTATAA
GGGAGAATTAGATGATGGGACACGAATCGCTGTCAAAAGAATGGAGGCTGGTATTGTTAGCAACAAAGCT

SEQ ID NO:24
TRK4 aa
AWINLTMINCNLAGPLPEFLGTMSSLEVLLLSTNRLSGPIPGTFKDAVLKMLWLNDQSGDGMSGSIDVVATMVSLT
HLWLHGNQFSGKIPVEIGNLTNLKDLSVNTNNLVGLIPESLANMPLDNLDLNNNHFMGPVPKFKATNVSFMSNSFC
QTKQGAVCAPEVMALLEFLDGVNYPSRLVESWSGNNPCDGRWWGISCDDNQKVSVINLPKSNLSGTLSPSIANLET
VTHIYLESNNLSGFVPSSWTSLKSLSILDLSNNNISPPLPKFTTPLKLVLNGNPKLTSNPPGANPSPNNSTTPADS
PTSSVPSSRPNSSSSVIFKPSEQSPEKKDSKSKIAIVVVPIAGFLLLVCLAIPLYIYVCKKSKDKHQAPTALVVHP

RDPSDSDNVVKIAIANQTNGSLSTVNASGSASIHSGESHLIEAGNLLISVQVLRNVTKNFSPENELGRGGFGVVYK
GELDDGTRIAVKRMEAGIVSNKA

SEQ ID NO:25

TRK5 3'

TGAGTCAGCATTTGTGTTGAATGGCAAGAATTAGGCTAACAAACCTCTTAACTTGGAAGGCAAAGGGTAACGATAG
CATTGGATGTGGCAAGAGGGGTTGAATACCTTTCATAGCTTGGCACAACAAAGTTTCATTCATAGAGATTTAAAAC
CGTCAAACATCCTTCTCGGGAGACGACATGAGAGCCAAGGTTGCAGATTTTGGTTTGGTTAAGAAATGCCCCTGAT
GGAAAGTATTCTGTGGAGACACGTTTGGCTGGAACTTTTGGCTATCTTGCACCTGAATACGCGGGTAAGCATTTCC
ACTCCTTGTATCATTACATCTTTTTTAACCGAGAACTGATTTGTATGCCCCTAGCTGTAAACTTTAGATCTTAGTA
AATTTTAAAATTATTCGATCATCTGCATTGCTAGGCATTCTGGTCTTCATTACATTTAGAGACATTTGGCACATAG
TGTTAATACTGAGGCAATCTCCATGCGTAAATGCTGCTTTGTCATTTTTATCGAACTCATGAACTATATGCTACTA
TTATAAAAGATAAGTTTCCATTTCGTGTGGATCAGCATCTGAATTATTATCCTATTACATCTCCTCTTTGTTACAG
CTACTGGACGAGTCACAACCAAAGTAGACGTTTACCTTTGGCGTTGTTTTGATGGAGATCATTACTGGTAGAAAAG
CT

SEQ ID NO:26

TRK5 5'

CTTTCTCCAACTCCTTCTGGTTGGTAAGTTCTAAGGCTTTCTGTTCTTGGACTAATGTATTTGTGACAAGTCTTCA
TCTACAGTAACTTCAATTAATCTTGATTCTCAATCTGTTTCTGGTTCTTTACCTTCTGANATTAGTCAACTTTCTA
ATCTTAAAACCCTTTCACTTCAAAAAAACAAACTTTCTGGCCCTTTACCTTCTTTTGCCAACATGTCAAAATTAGC
TGATCTTTTCTTGGACAATAACCAATTCACTTCTGTTCCTCAAGATTTCCTTTTGGGGGTTCCTAGTTTAGTAACT
TTAAGCATTANTGAAAATGCGGGACTCTCTCCTTGGCAAATACCTATGTATTTAACTGANAGTACCAAATTTGGGA
TCTCTATATGCTAGTAATGCAAGTATT

SEQ ID NO:27

TRK5 5' aa

LSPTPSGWSTSKPFCSWTNVICDKSSSTVTSINLDSQSVSGSLPSDISQLSNLKTLSLQKNKLSGPLPSFANMSKL
ADLFLDNNQFTSVPQDFLLGVPSLVTLSISENAGLSPWQIPMYLTESTKFGISIC..CKY

SEQ ID NO:28

TRK6 3'

GCTTATGACAGTCATTGATCCAACTCTAGACGTGAAAGATGAGATAACCGAGAGCATTTCCACCTTAGCTGAACTT
GCTGGTCATTGCACTGCAAGAGAACCTGGTCAACGGCCAGATATGGGCCACGCTGTAAACGTGCTGTCCCCACTTG
TTGAGAAATGGAAGCCTCTTGAGGATGATCCAGCAGGACTATTGTGGTATCGACTACAGTCTTCCCCTCAATCAAA
TGGTCAAGGGTTGGCAAGAATCGGAAGGAAAAGACTTAAGTTACGTGGATCTCGAGGACAGTAAGGGCAGTATCCC
AGCAAGACCAACTGGATTTGCAGATTCATTTACATCAGCTGATGGTAGATAATGGAGGTACTTCTATGTAGTAGAT
GTAGATATCAATTTTCTTTGTATTGTATTGAGATTTTGATCGTATTTTCCACGTGCCTTCGCTCATTTCTCCCCCT
TCAATGTGAATGTATTAGAAATTTAAACTATGTGTAGCCTCAGTTCCTTCTGTAGATATAAAATAGCGGTGAAGGA
GAACTATACATCGATCAGTTGAACTCCTCGCTCAGTCACTATTTTCATCTTCTACTATGGTGAGATTTAAGAGCAT
TTTTTTCACCTTTGCCTATTTTCGTCGTTTAGTTGTGCTGCTTCTGAAGCTCTGTGCTGAATACATACTGAGCGGT
GAAGTAGCCCGGTATATAGAATATGTCGTTTGATTCGAAGAATCTAACCATATAGCTTCTTCCTTCTGCTCAAATA
GGGCAATACTTGGAGAAGATAACCCTTATGTGTCCTCATCATTTGTTGTTTTGAGAAAAAAGTATGATATATGTTG
CCTTAGACTCTTCGAAAAGGTATTGTCAT

SEQ ID NO:29
TRK7 3'

AAGCTTTCGATTCTCCCTATATGTCTCTCATGTTAACTAATATAGCTTGATAAACCTAGTAAATTCTTCATACTTT
GTGTATATAGTGATATCGAAACGTGAGATGCAGCCTTTATCCGCCTTGTTCTTTTTTCTTGGCACTCTACTTGTTC
TGTTACTTCTCATATTTATCTTATGCAAGTTTTTCAAACCAGGAAAACTGACAGCTTTGATTACTAGCACATTTAA
GCCTCTTCCAGGTTTTGTTTTGTTTCGAATTCATGTTACTTTGAGTATCTTATTCGTTATTTTAAAATATGTCTTT
TTTCGGATGATACAGAGATGAGTAATGCTGTTAGTGTACATCTTCATACAATAAGCTACTTTAACTTTCATACATT
GGAGAAAGCTACGAAGAATTTTCACTCGGATAACCTCCTTGGATGTGGTGGATTTGGTCCGGTTTTCCTGGTAAGG
TCAATGTGTCTTAACGAGTTGTAAAATCCGTTTTTATAATTAGATGCAGAGGTTAAGAATTGGCCCTAGTGTTTTT
TCTTAGGGGAAGTTAGGAGATGGACAATTAGTTGCTGTCAAGAAGTTGTCTGTTGACAAATCGCAGCAAGGGGACC
GGGAGTTTCTTGCTGAGGTACGGATGATAACAAGTATACAACACAAGAACCTTGTCCGCCTTCTTGGTTGTTTGTT
CAAAAGGGGGATCAGAGGCCTTCTCGTGTAACGAATAACATGAAGAATAAGGAGGCTTGGGACCAAATACTATATT
GGTATTTAT